

Amendments to the Drawings

The attached sheets of drawings include changes to FIGS. 1-11 as needed to answer the issues raised on form PTO 948. These sheets replace all the original sheets of drawings.

Attachment: Replacement sheets

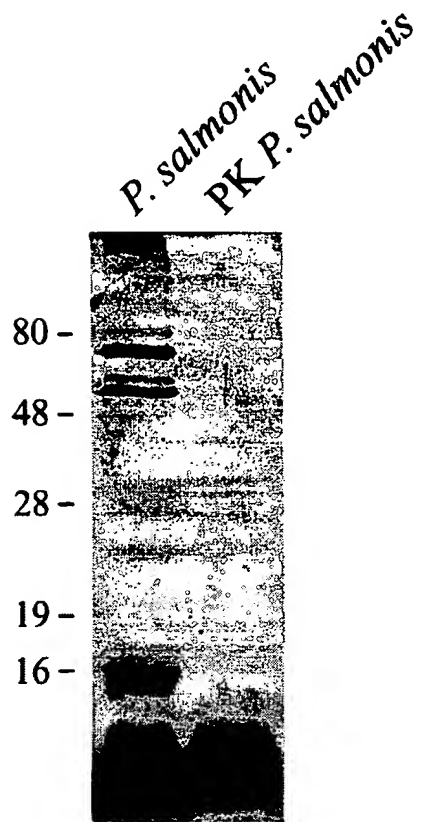
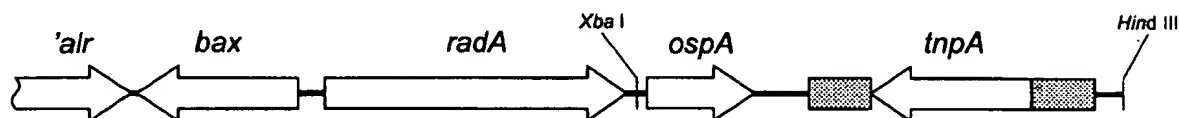
FIGURE 1. WESTERN BLOT ANALYSIS OF *P. SALMONIS*

FIGURE 2

A. ORF's in the region of the *ospA* gene from *P. salmonis*



B. DNA sequence of *ospA* gene from *P. salmonis* (SEQ ID:1)

ATGAACAGAGGATGTTTGCAGGTAGTAGTCTAATTATTATCAGTGTGTTTTAGTTGGCTGTGCCCAGA
 ACTTTAGTCGTCAAGAAGTCGGAGCTGCGACTGGGGCTGTTGTTGGCGGTGTTGCTGGCCAGCTGTTTGG
 TAAAGGTAGTGGTCGAGTTGCAATGGCCATTGGTGGTGCTGTTTTGGGTGGATTAAATTGGTTCTAAAATC
 GGTCAATCGATGGATCAGCAGGATAAAATAAAGCTAAACCAGAGTTTGGAAAAGGTAAAAGCAGGGCAAG
 TGACACGTTGGCGTAATCCAGATACAGGCAATAGTTATAGTGTTGAGCCAGTGCGTACTTACCAGCGTTA
 CAATAAGCAAGAGCGTCGCCAGCAATATTGTGAGAATTTACAGCAAAGGCGATGATTGCAGGGCAGAAG
 CAAGAGATTTACGGCACTGCATGCCGGCAACCGGATGGTCGTTGGCAAGTCATTTCAACAGAAAAA

Amino acid sequence of OspA protein (SEQ ID:2)

MNRGCLQGSSLIISVFLVGCANFSRQEVGAATGAVVGGVAGQLFGKSGRVAMAIGGAVLGGLIGSKI
 QSMDQQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQYCREFOQKAMIAGQK
 QEIYGTACRQPDGRWQVISTEK

C. Sequence alignment of the OspA proteins of *P. salmonis* and *R. prowazekii*

| | | | | | |
|------------------------|--|-------------------------|--------------|----------------------------|-----|
| | 10 | 20 | 30 | 40 | |
| <i>P. salmonis</i> : | MNRGCLQGS | SLIITISV | ---FIVGCA | ---QNFSRQEVGAATGAVVGGVAGQL | |
| <i>R. prowazekii</i> : | MKLLSKIMTIALAASMLQACNGQSGMNRKQGTGILLGAGGAILGSQ | | | | |
| | 10 | 20 | 30 | 40 | |
| | 50 | 60 | 70 | 80 | 90 |
| <i>P. salmonis</i> : | FGKCSGRVAMAI | GGAVLGGGLIGSKI | QSMDQQDKIKLN | ---QSLKVKFA | |
| <i>R. prowazekii</i> : | FGQCKGQLVGVGVGAILGCAVLGGQIGASMDQDRRLLELTSCRALESAPS | | | | |
| | 50 | 60 | 70 | 80 | 90 |
| | 100 | 110 | 120 | 130 | 140 |
| <i>P. salmonis</i> : | GQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQYCREFOQKAMIAGQKQ | | | | |
| <i>R. prowazekii</i> : | GSNIETWRNPDGNHGYVTPNKTYR | ---NSAGQYCREYTCIVILGGKQ | | | |
| | 100 | 110 | 120 | 130 | 140 |
| | 150 | 160 | | | |
| <i>P. salmonis</i> : | EITGTACRQPDGRWQVISTEK | | | | |
| <i>R. prowazekii</i> : | KIYGTACRQPDGRWQVVN | | | | |
| | 150 | | | | |

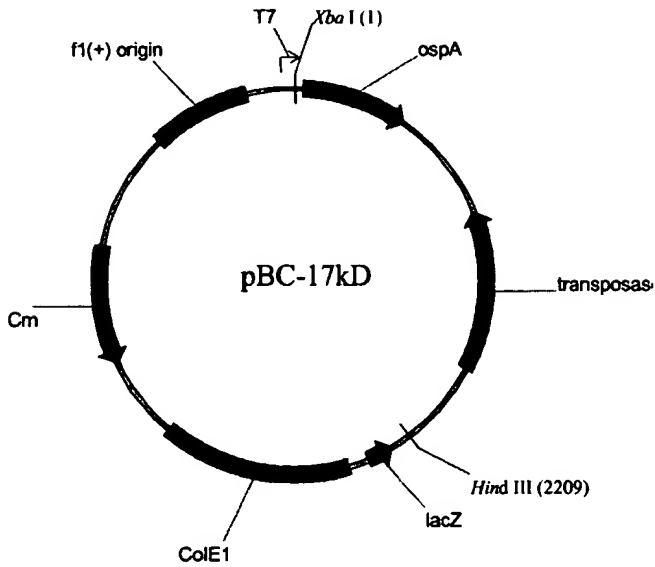
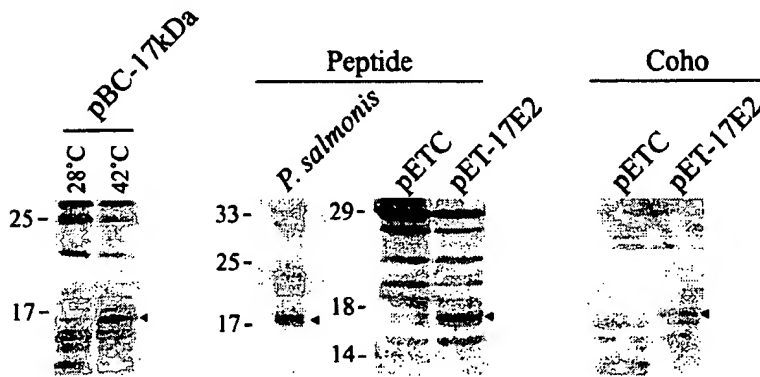
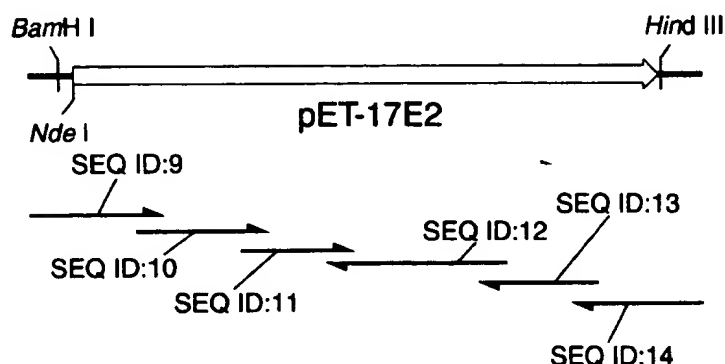
FIGURE 3**A. Map of plasmid pBC-17kDa encoding the *ospA* ORF.****B. Western blot analysis of OspA expression.**

FIGURE 4.**A. Strategy for construction of the *E. coli* codon optimized *ospA* gene.****B. Oligonucleotide #1 (SEQ ID:9)**

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATC
ATTATCTCTGTTTTCTGGTGGGTTGCGCCAGAACTTCAG

Oligonucleotide #2 (SEQ ID:10)

TGGGTTGCGCCAGAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGC
CGGCCAGCTGTTTCGGTAAAGGCTCTGGTCGTGTGGCGATG

Oligonucleotide #3 (SEQ ID:11)

AAAGGCTCTGGTCGTGTGGCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCG
GTCAGAGCATGGACCAGCAGGATA

Oligonucleotide #4 (SEQ ID:12)

GTTCCACAGAGTAGCTGTTACCGGTGTCCGATTACGCCAACGAGTAACCTGGCCGGCTTTCACTTTTTTC
CAGAGACTGGTTCAGTTTGATTTTATCCTGCTGGTCCATGCTCTGACC

Oligonucleotide #5 (SEQ ID:13)

GGTGCCGTAGATTTCTGTTTCTGACCTGCGATCATGGCTTTCTGCTGAAATTCGCGGCAGTACTGCTGA
CGCGTTCTGTTTGTTGTAACGCTGGTAGGT

Oligonucleotide #6 (SEQ ID:14)

CGTCTCTCGTCCTGGTCCGAATTCAGATAAGCTTATTTTTCGGTGCTAATCACCTGCCAGCGGCCATCC
GGCTGACGGCACGCGGTGCCGTAGATTTCTGTTTCTGAC

C. DNA sequence of *E. coli* optimized *ospA* gene, 17e2 (SEQ ID:3)

ATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCTGGTGGGTTGCGCCAGAACT
TCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTTCGGTAA
AGGCTCTGGTCGTGTGTGTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCGGT
CAGAGCATGGACCAGCAGGATAAAAATCAAACCTGAACCACTCTCTGGAAAAAGTGAAAGCCGGCCAGGTTA
CTCGTTGGCGTAATCCGGACACCGGTAACAGCTACTCTGTGGAACCGGTTTCGCACCTACCAGCGTTACAA
CAAACAGGAACGCCGTGACGAGTACTGCCGCGAATTTTCAGCAGAAAGCCATGATCGCAGGTGAGAAACAG
GAAATCTACGGCACCGGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

FIGURE 5

A. Amino acid sequence of optimized OspA protein, 17E2, (SEQ ID:4).

MRGCLQGSSLI I I SVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKSGSRVSM AIGGAVLGGLIGSKIG
QSM DQQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQQYCREFQQKAMIAGQKQ
EIYGTACPQPDGRWQVISTEK

B. DNA sequence of c17e2 *ospA* construct with N-terminal fusion partner (SEQ ID:5).

ATGTCAGTTGAATTCTACAACCTCTAACAATCAGCACAAACAACTCAATTACACCAATAATCAAAATTA
CTAACACATCTGACAGTGATTTAAATTTAAATGACGTAAAAGTTAGATATTATTACACAAGTGATGGTAC
ACAAGGACAACTTTCTGGTGTGACCATGCTGGTGCAATTATTAGGAAATAGCTATGTTGATAACACTAGC
AAAGTGACAGCAAACTTCGTTAAAGAAACAGCAAGCCCCAACATCAACCTATGATACATATCTGGATCCGT
CTCATATGCGTGGTTGCCGTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCCTGGTGGGTTGCGCCA
GAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTT
GGTAAAGGCTCTGGTCTGTGTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAA
TCGGTCAGAGCATGGACCAGCAGGATAAAATCAAACCTGAACCAAGTCTCTGGAAAAAGTGAAAGCCGCCA
GGTTACTCGTTGGCGTAATCCGGACACCGGTAACAGCTACTCTGTGGAACCGGTTTCGCACCTACCAGCGT
TACAACAAACAGGAACGCCGTCAGCAGTACTGCCGCGAATTTTCAGCAGAAAGCCATGATCGCAGGTCAGA
AACAGGAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

C. Amino acid sequence of C17E2 OspA construct with N-terminal fusion partner (SEQ ID:6).

MSVEFYNSNKS AQTNSITPI I KITNTSDSDLNLNDVKVRYYYTSDGTQGQTFWCDHAGALLGNSYVDNTS
KVTANFVKETASPTSTYDYLDP SHMRGCLQGSSLI I I SVFLVGCAQNFSRQEVGAATGAVVGGVAGQLF
GKSGSRVSM AIGGAVLGGLIGSKIGQSM DQQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQR
YNKQERRQQYCREFQQKAMIAGQKQEIYGTACPQPDGRWQVISTEK

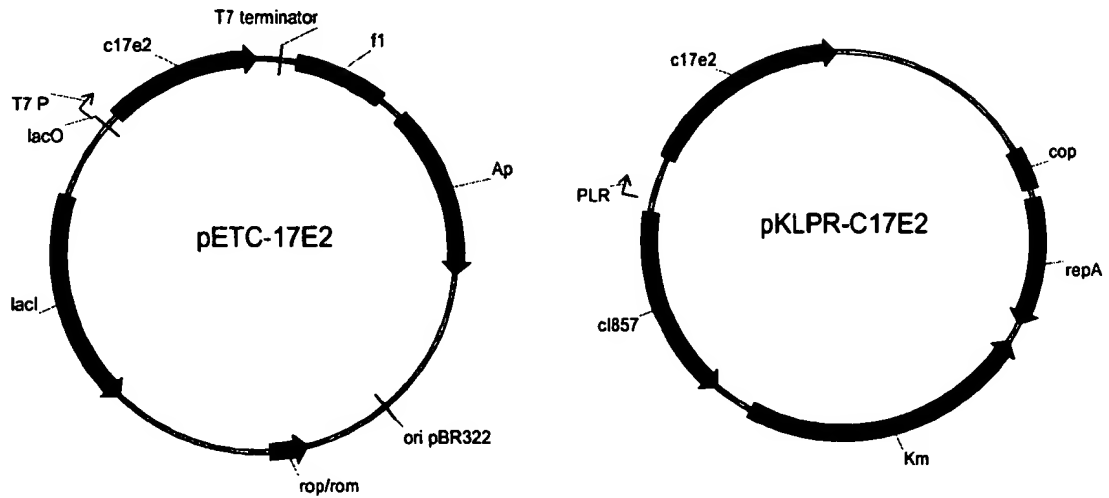
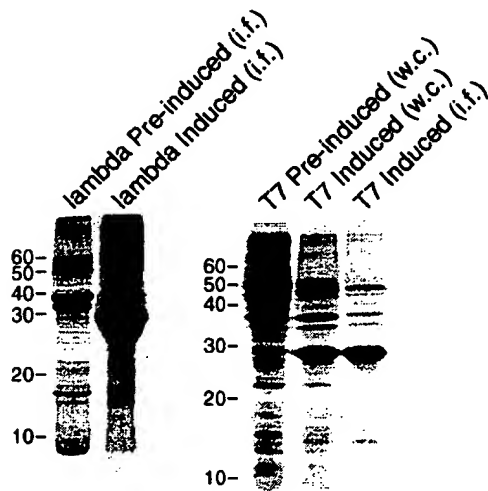
FIGURE 6**A. Expression vectors encoding the optimized *ospA* fusion constructs****B. SDS-PAGE analysis of C17E2 expression.**

FIGURE 7

Map of the *ospA*-fusion construct encoding a C-terminal fusion partner under T7 promoter control.

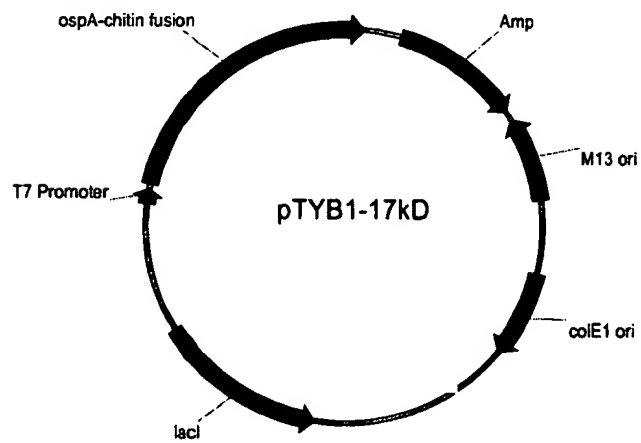
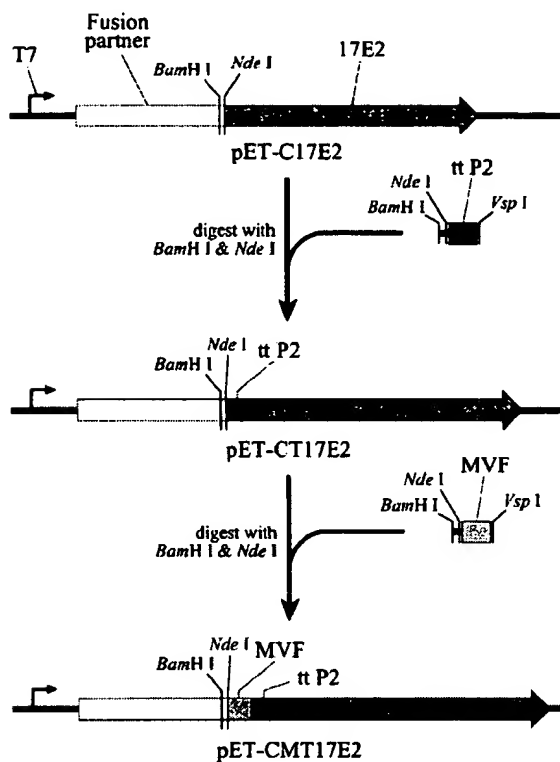


FIGURE 8

A. CLONING STRATEGY FOR OSPA TCE FUSION PROTEIN CONSTRUCTS.



B. (a) Nucleotide sequence of the tt P2 oligonucleotide (SEQ ID:17)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCAGTACATTAAAGCAAACCTCTAAATTCATC
GGTATTACCGAACTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(b) Nucleotide sequence of the MVF oligonucleotide (SEQ ID:18)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCTGTCTGAAATCAAAGGTGTTATCGTTCAT
CGTCTGGAAGGCGTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(c) Amino acid sequence of the tt P2 TCE (SEQ ID:19)

QYIKANSKFIGITEL

(d) Amino acid sequence of the MVF TCE (SEQ ID:20)

LSEIKGVIVHRLEGV

FIGURE 9

Coho salmon antibody titres against OspA-fusion protein candidate vaccines.

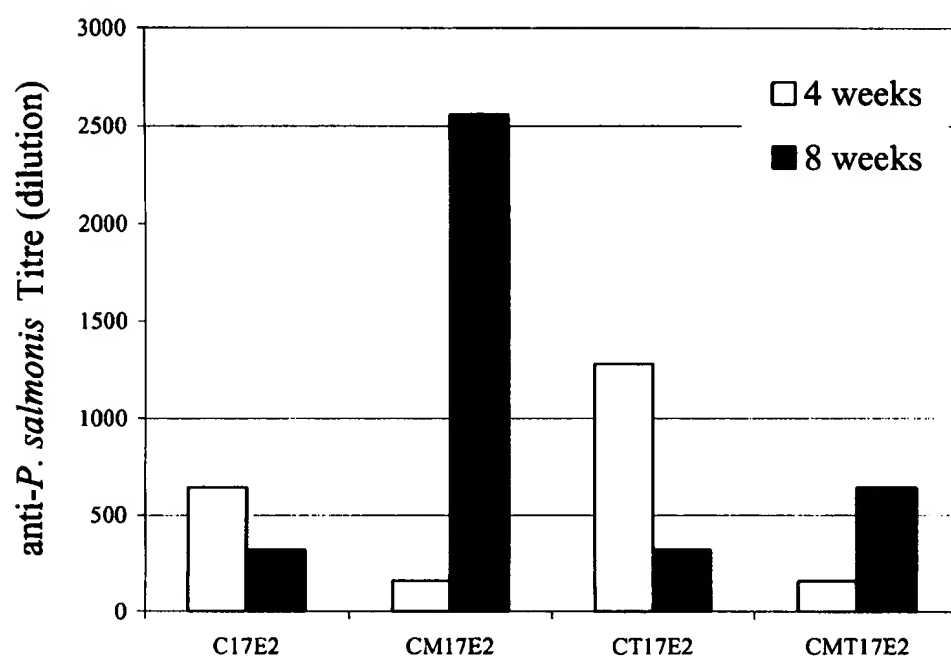


FIGURE 10

Whole lymphocyte proliferative response to OspA-fusion proteins in Atlantic salmon.

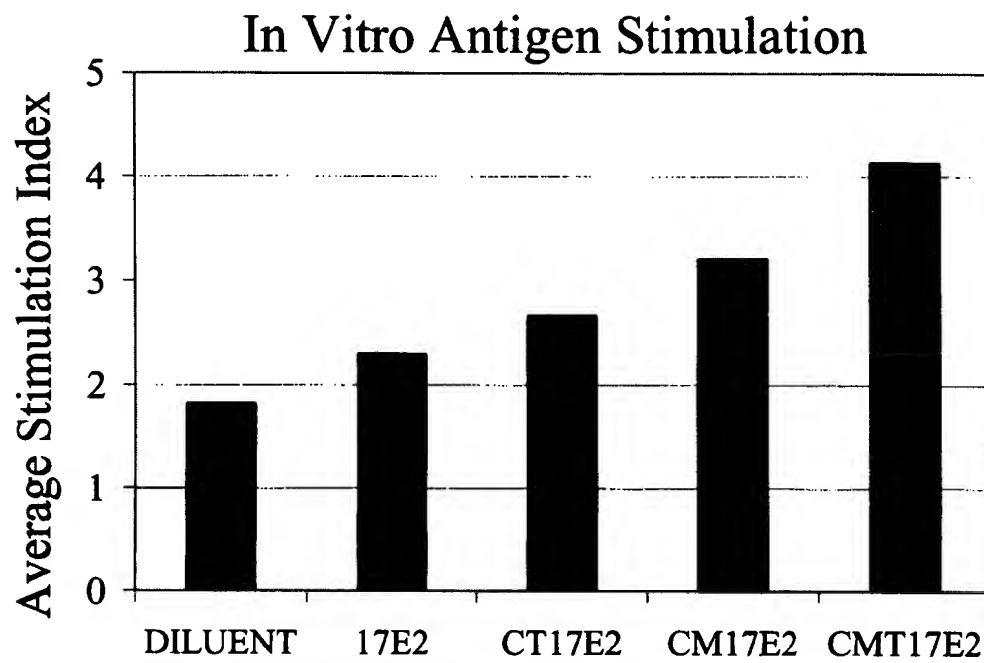


FIGURE 11**Vaccine trial in coho salmon of OspA fusion proteins.**